REMARKS/ARGUMENTS

Claims 1-14 and 21-24 are active. Claims 15-20 were cancelled without prejudice. Claim 12 was made dependent on claim 1 and rewritten to incorporate the step (1) of claim 9 and the step (2) of claim 11. No new matter is believed to have been added. Favorable consideration of this Amendment and allowance of this application are now respectfully requested.

Restriction/Election

The Applicants previously elected with traverse **Group I** claims 1-4, directed to producing a heterologous RNA of interest. The requirement has been made FINAL. The Applicants respectfully request that the claims of the nonelected group(s), or which are directed to other withdrawn subject matter, which depend from or otherwise include all the limitations of an allowed elected claim, be rejoined upon an indication of allowability for the elected claim, see MPEP 821.04.

Objection

Claim 12 was objected to as being improperly dependent. This objection is now moot.

Rejections—35 U.S.C. §103

The Applicants indicate that the claimed subject matter was commonly-owned at the time of invention.

Rejection—35 U.S.C. §103(a)

Claims 1, 2, 5, 7, 8, 11 and 21-23 were rejected under 35 U.S.C. §103(a) as being unpatentable over Bonnefoy, et al., Meth. Enzymol. 350: 97-111 (2001), in view of Kaisho, et al., Yeast 5: 91-98 (1989). The cited prior art does not disclose all the elements of the claimed method, suggest the combination of elements of the claimed method, or provide a reasonable expectation of success for producing a heterologous RNA of interest using this method. Since Bonnefoy and Kaisho are the primary reference for each obviousness rejection below, a detailed evaluation of their teachings is provided below.

- *Bonnefoy*

As discussed in the response to the first Office Action, Bonnefoy *et al.*, generally discloses genetic transformation of mitochondria in the yeast *S. cerevisiae* and pertains to how to deliver exogenous DNA into mitochondria and to generate *S. cerevisiae* mitochondrial recombinants having incorporated targeted mutations in a mitochondrial gene or having inserted new genes into mitochondrial DNA (mtDNA) via homologous recombination (see introduction, page 97).

Bonnefoy *et al.*, teaches (pages 101-109) a first method for making a mitochondrial recombinant of *S. cerevisiae*, comprising:

-transforming the mitochondria of yeast cells lacking mitochondrial DNA (rho^0 cells) by bombardment of the yeast cells with a mixture of a plasmid carrying the nuclear marker and a nuclear replication origin and a plasmid carrying the mitochondrial DNA of interest, precipitated onto microparticles, and

-identifying mitochondrial transformants that have incorporated the DNA of interest (synthetic rho^-) by mating with a tester strain (rho^+ mit^-) to detect respiring diploids (marker rescue) and repeat mating until pure stable synthetic rho^- clones are obtained (figure 1) or by

scoring for expression of a mitochondrial transformation reporter gene *ARG8*^{m} that allows growth without arginine (Arg+ prototrophy),

-mating a rho+ recipient strain with a deletion in the region of interest with a synthetic rho^{-} (donor transformant) that carries the wild-type information and that can recombine with the rho+ recipient mutant and identifying rho+ recombinants, for example by marker rescue (figure 2).

Bonnefoy *et al.*, teaches (pages 109-110) a second method (direct method) for making a mitochondrial recombinant of *S. cerevisiae*, comprising:

-transforming the mitochondria of yeast cells comprising mitochondrial DNA (rho+ cells) by bombardment of the yeast cells with plasmid or linear DNA carrying the mitochondrial DNA of interest, precipitated onto microparticles, and

-identifying rho+ mitochondrial recombinants that have integrated the transforming DNA into the rho+ mtDNA by phenotypic selection: restoration of growth on a nonfermentable carbon source (YPEG medium) or expression of a mitochondrial transformation reporter gene *ARG8*^{m} that allows growth without arginine (Arg+ prototrophy).

Bonnefoy is cited as teaching the transformation of *S. cerevisiae* mitochondria lacking mitochondrial DNA (rho^{0} cells) with a mitochondrial transcription vector comprising a mitochondrial transformation reporter gene (*ARG8*^{m}).

Bonnefoy discloses the transformation of yeast mitochondria lacking mitochondrial DNA with a plasmid carrying the mitochondrial DNA of interest, *i.e.* a mutated mitochondrial gene to replace with the wild-type one or a new gene to insert into mitochondrial DNA (page 103, step (b) of "preparation of microprojectiles and precipitation of DNA", introduction, page 97, page 105, first paragraph of chapter "*integration of altered mtDNA sequences by Homologous Double Crossovers*").

Bonnefoy discloses (2) the identification of the yeast mitochondrial transformants that have incorporated the DNA of interest (page 101). Bonnefoy teaches that the transformants may be identified by mating with a tester strain (rho$^+$ mit$^-$) to detect respiring diploids (marker rescue; figure 1) or by scoring for expression of a mitochondrial transformation reporter gene, such as *ARG8*^{m} that allows growth without arginine (Arg+ prototrophy: pages 98-99).

However, Bonnefoy does not disclose or suggest : (1) transforming the mitochondria of yeast cells lacking mitochondrial DNA (rho^0 cells) with a mitochondrial transcription vector comprising a mitochondrial transformation reporter gene (*ARG8*^{m}) and a DNA encoding a heterologous RNA of interest.

Bonnefoy does not disclose or suggest: (3) culturing the yeast mitochondrial transformants selected in (2); (4) isolating the mitochondria from the yeast mitochondrial transformants obtained in (3), and (4) extracting and purifying the heterologous RNA of interest from said mitochondria.

As mentioned by the Examiner in the first and second Office Actions, Bonnefoy does not disclose or suggest a process of producing a heterologous RNA of interest. As discussed already by the Applicant in the response to the first Office Action, Bonnefoy does not suggest a process of expressing heterologous RNA in mitochondria lacking mitochondrial DNA.

- *Kaisho*

Kaisho was relied upon as teaching, especially on pages 91, 94-96 and on figure 3, transforming yeast mitochondria lacking mitochondria DNA with a plasmid comprising a gene which is expressed to produce RNA. The DNA encoding the RNA was under the control of a promoter and a terminator that are functional in yeast mitochondria, since the RNA was successfully produced in yeast mitochondria. The mitochondria were isolated, and then the RNA was isolated from the mitochondria.

However this is not true for the following reasons.

Kaisho relates to the use of *S. cerevisiae* for producing mammalian proteins by recombinant DNA techniques (introduction page 91, first column). Kaisho is directed to solving the problem of increasing recombinant protein production in *S. cerevisiae* (introduction page 91, first column, middle of first paragraph and end of 2nd paragraph).

Kaisho teaches that the productivity of human lysozyme, human epidermal growth factor and other foreign proteins is increased in respiratory-deficient mutants of *S. cerevisiae* (rho^- cells) and that the rho^- mutation is one of the efficient techniques for increasing the production of many useful proteins by yeast (end of introduction page 91 and discussion, page 96, 2nd column, last paragraph to page 97, first column, beginning of first paragraph).

Kaisho discloses expression plasmids comprising: a "*DNA encoding a modified signal peptide of c-lysozyme and the mature* ***protein*** *of h-lysozyme*" downstream from the *GLD*, *PHO5* or *GAL10* promoter (page 91, 2nd column, end of last paragraph) or the *PHO5* gene encoding an acid phosphatase, downstream of the HIS5 promoter (pages 92, 1st column and page 96, 1st column). The production of the recombinant proteins is measured by assaying their enzyme activity (page 92, 2nd column middle paragraph; figures 1, 2 and Tables 2 and 3).

Therefore, Kaisho teaches expression plasmids comprising a gene which is expressed to produce a protein of interest. Kaisho does not disclose or suggest a plasmid comprising a gene which is expressed to produce an RNA of interest.

Kaisho mentions (page 92, 2nd column, paragraph "T*ransformation*") that *S.cerevisiae* was transformed with the expression plasmids as described by Ito, J. Bacteriol., 1983, 153, 163-168 *(attached)*. Ito describes a yeast transformation method that uses alkali metal ions

like Li+. Ito teaches that the alkali metal ions alter yeast cell membrane so that plasmid DNA can be incorporated into yeast cells (see introduction of Ito, page 163, first column).

Therefore, Kaisho teaches transforming yeast cells with a plasmid comprising a gene which is expressed to produce a protein of interest. Kaisho does not disclose or suggest transforming yeast mitochondria with a plasmid comprising a gene which is expressed to produce a RNA of interest.

Kaisho teaches (page 93) the transformation of respiratory-deficient mutants of yeast (rho$^-$ cells). Rho$^-$ cells are yeast strains comprising a large deletion of the mitochondrial genome (see the definition page 6 of the specification of the present application). The definition page 6 of the specification mentions that "*The conserved region of the mitochondrial genome* (of the rho$^-$ cells) *is reiterated, directly or a palindrome, so as to reconstitute a mass of mitochondrial DNA equivalent to that that exists in a wild-type yeast cell*". Therefore rho$^-$ cells do not lack mitochondrial DNA. The yeast strains lacking mitochondrial DNA are the rho^0 strains (see page 5 of the specification of the present application) which are different from the rho$^-$ cells.

Therefore, Kaisho does not disclose or suggest transforming yeast mitochondria lacking mitochondria DNA. Kaisho teaches transforming cells of a respiratory-deficient mutant of yeast comprising a large deletion of the mitochondrial genome (rho$^-$ cells).

To understand why the recombinant h-lysozyme protein production was increased in the rho$^-$ cell transformants compared to the rho$^+$ cell transformants, Kaisho has isolated total RNAs from the cell transformants (page 92, 2nd column, last paragraph, page 94, 2nd column, last paragraph and figure 3) and analysed the expression of the h-lysozyme mRNA by northern blot using a labelled fragment of the h-lysozyme gene as a probe (figure 3).

Kaisho does not disclose or suggest that the h-lysozyme mRNA was produced in yeast mitochondria. Kaisho teaches only that h-lysozyme mRNA was produced in the yeast cells and that the rho$^-$ mutation increases the rate of transcription or the stability of this mRNA.

Furthermore, Kaisho does not disclose or suggest that the mitochondria were isolated and then the RNA was isolated from the mitochondria. Kaisho teaches only that total RNA was isolated from the yeast cell-transformants. In conclusion, Kaisho teaches that the rho$^-$ mutation is one of the efficient techniques for increasing the production of many useful proteins by yeast.

Kaisho does not disclose or suggest a process of producing a heterologous RNA of interest in the mitochondria of yeast cells. Moreover, Kaisho does not disclose or suggest a process of producing a heterologous RNA of interest comprising: transforming yeast mitochondria lacking DNA with a plasmid comprising a gene which is transcribed to produce a heterologous RNA of interest.

- *Non-obviousness of the claimed method over Bonnefoy et al. in view of Kaisho et al.*

The ordinary skilled artisan is a specialist in recombinant DNA technology and gene expression in prokaryotic and eukaryotic expression systems. The ordinary skilled artisan desiring to produce a heterologous RNA of interest would have found no motivation to combine the teaching of Bonnefoy teaching transformation of *S. cerevisiae* mitochondria with the teaching of Kaisho teaching transforming rho$^-$ cells of yeast (which do not lack mitochondrial DNA) with a plasmid comprising a DNA encoding a protein of interest, because these documents do not suggest the expression of a heterologous RNA in the mitochondria of yeast cells.

The Examiner considers (page 5 of the present Office Action) that the skilled artisan desiring to produce a heterologous RNA of interest would find some motivation to combine the teaching of Bonnefoy with the teaching of Kaisho because Bonnefoy state that genetic manipulation of *S. cerevisiae* mitochondria are amenable to *in vivo* experimental analysis and should provide a useful model for other system.

However, this is not true. As discussed already in pages 10 and 11 of the response to the first Office Action, a reference should always be considered for everything it would have fairly taught a person having ordinary skill in the art.

Bonnefoy teach that the fact that there is no specific replication origin sequence in rho$^-$ mitochondrial DNAs (mtDNAs) is advantageous in creating mitochondrial transformants containing defined mtDNAs, as rho^0 yeast strains (entirely lacking mitochondrial DNA) can be transformed with bacterial plasmid DNAs that subsequently propagate as synthetic rho$^-$ molecules (last sentence of page 99 to end of first paragraph of page 100). Bonnefoy points out that these features of *S. cerevisiae* mitochondrial genetic system allow DNA introduced from outside the cell (bacterial plasmid DNA) to be propagated within the mitochondria as a plasmid (synthetic rho$^-$ molecule), and the plasmid-borne mitochondrial sequences to recombine homologously with complete rho+ mtDNA (last paragraph of page 100). Therefore, Bonnefoy teaches how the mitochondrial genome can be manipulated using genetic transformation procedures.

In the "*Concluding Remarks*" page 111, Bonnefoy states that "*the methods developed for manipulation of the S. cerevisiae mitochondrial genome should provide a useful model for other* systems. Indeed, in Chlamydomonas, another single-celled eukaryote.....*Thus, as appropriate selectable markers are developed for other species, it seems likely that their mitochondrial genomes will become amenable to in vivo experimental analysis*."

As discussed above, Bonnefoy teaches only how the mitochondrial genome can be manipulated using genetic transformation procedures. Even if the concluding remarks suggest that the methods developed for manipulating *S. cerevisiae* mitochondrial genome would be usable for other single-cells eukaryote species, nothing in Bonnefoy would have motivated the artisan of ordinary skill to produce a RNA of interest in the mitochondria of yeast cells lacking mitochondrial DNA.

Kaisho teaches that the rho$^-$ mutation which increases the rate of transcription of the gene in an expression plasmid or the stability of the mRNA in the rho$^-$ cells is one of the efficient techniques for increasing the production of many useful proteins by yeast.

Kaisho suggest only to use rho$^-$ yeast cells transformed with an expression plasmid to produce a heterologous RNA of interest. Kaisho does not suggest using rho^0 yeast cells to produce RNA or producing the heterologous RNA of interest in the mitochondria of yeast cells.

The prior art teaches yeast expression system that uses yeast cells transformed with an expression plasmid or recombinant yeasts having integrated the gene of interest in their genome to produce a protein of interest (see for example, pages 165-166 of Fincham, Microbiol. Rev., 1989, 53, cited by the Examiner). As mentioned in the present application (page 2, line 24 to page 3, line 12 of the specification), even if the systems described for the production of proteins were transposed to the production of a RNA of interest, it would not be possible to specifically produce the RNA of interest, but only a mixture of endogenous yeast RNAs in which the proportion of exogenous RNA of interest in the total yeast RNA fraction is increased.

Given the teaching of the prior art and the level of ordinary skill in the relevant art, the skilled artisan would have had no clue of the potential utility of yeast mitochondria to produce a heterologous RNA of interest. Therefore, by combining the teaching of Bonnefoy

with the teaching of Kaisho the artisan of ordinary skill would not arrived at the claimed invention with reasonable expectation of success.

On the other hand, discussed in the response to the first Office Action, the claimed method surprisingly allows one to produce a RNA of interest in large amounts, for a low cost, and in a form which is stable and can be readily isolated insofar as the only RNAs produced in the mitochondria of the synthetic rho$^-$ yeast strain are those which are encoded by the DNA used for the transformation (as mentioned page 4, lines 20-25 and page 7, line 31 to page 8, line 17 of the specification and demonstrated in the example page 26, line 28 to page 27, line 4 and in figure 4). For all of these reasons, this rejection and those below which also rely on the combination Bonnefoy and Kaisho cannot be sustained

Rejection—35 U.S.C. §103(a)

Claim 3 was rejected under 35 U.S.C. §103(a) as being unpatentable over Bonnefoy, et al., Meth. Enzymol. 350: 97, in view of Kaisho, et al., Yeast 5: 91, and further in view of Dziembowski, et al., J.B.C. 278:1603.

This rejection is not sustainable over the combination of Bonnefoy and Kaisho for the reasons considered above. Furthermore, Dziembowski does not suggest the elements missing from the two primary references, such as expression of a heterologous RNA in yeast mitochondria lacking mitochondrial DNA as discussed already in the response to the previous Office Action. Accordingly, this rejection cannot be sustained.

Rejection—35 U.S.C. §103(a)

Claim 4 was rejected under 35 U.S.C. §103(a) as being unpatentable over Bonnefoy, et al., Meth. Enzymol. 350: 97, in view of Kaisho, et al., Yeast 5: 91, and further in view of Komiya, et al., J.B.C. 269:30893 and Hwang, et al., J. Virol. 74:4074. This rejection cannot

be sustained for the reasons discussed above. Komiya and Hwang were relied upon, respectively, as disclosing "viral RNAP integrated into the genome of *Pichia*" and "using a mitochondrial targeting signal for cytosolic import". However, these secondary references do not suggest the elements missing from Bonnefoy and Kaisho. Accordingly, this rejection cannot be sustained.

Rejection—35 U.S.C. §103(a)

Claim 6 was rejected under 35 U.S.C. §103(a) as being unpatentable over Bonnefoy, et al., Meth. Enzymol. 350: 97, in view of Kaisho, et al., Yeast 5: 91, and further in view of Anziano, et al., PNAS 88:5592. Anziano was cited as teaching the COXII gene as a reporter, but does not remedy the deficiencies of Bonnefoy or Lisowsky does not suggest the elements missing from the two primary references, such as expression of a heterologous RNA in yeast mitochondria lacking mitochondrial DNA. Accordingly, this rejection cannot be sustained.

Rejection—35 U.S.C. §103(a)

Claims 9 and 10 were rejected under 35 U.S.C. §103(a) as being unpatentable over Bonnefoy, et al., Meth. Enzymol. 350: 97, in view of Kaisho, et al., Yeast 5: 91, and further in view of Fincham, Micro. Rev. 53:148. Furthermore, Fincham does not suggest the elements missing from the two primary references, such as expression of a heterologous RNA in yeast mitochondria lacking mitochondrial DNA as discussed already in the response to the previous Office Action. Accordingly, this rejection cannot be sustained.

Rejection—35 U.S.C. §103(a)

Claim 13 was rejected under 35 U.S.C. §103(a) as being unpatentable over Bonnefoy, et al., Meth. Enzymol. 350: 97, in view of Kaisho, et al., Yeast 5: 91, and further in view of Kim, et al., Canc. Res. 57:3115.

Kim was relied upon for teaching the lysing and centrifuging steps of claim 13. However, it does not teach the elements missing from Bonnefoy and Kaisho, such as expression of a heterologous RNA in yeast mitochondria lacking mitochondrial DNA as discussed already in the response to the previous Office Action. Accordingly, this rejection cannot be sustained.

Rejection—35 U.S.C. §103(a)

Claim 14 was rejected under 35 U.S.C. §103(a) as being unpatentable over Bonnefoy, et al., Meth. Enzymol. 350: 97, in view of Kaisho, et al., Yeast 5: 91, and further in view of Dziembowski, et al., J.B.C. 278:1603. and diRago, et al., J.B.C. 263:12564.

Dziembowski has been addressed above, it in combination with diRago were also relied upon for disclosing particular buffers for lysing cells and organelles of claim 14. However, these references do not teach the elements missing from Bonnefoy and Kaisho, namely expression of heterologous RNA using "yeast cells lacking mitochondrial DNA". Furthermore, di Rago does not suggest the elements missing from the two primary references, such as expression of a heterologous RNA in yeast mitochondria lacking mitochondrial DNA as discussed already in the response to the previous Office Action. Accordingly, this rejection cannot be sustained.

Conclusion

In view of the amendments and remarks above, the Applicants respectfully submit that this application is now in condition for allowance. An early notice to that effect is earnestly solicited.

Respectfully submitted,

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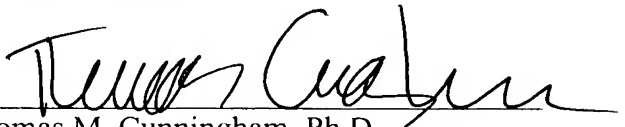

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